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Doll, Hans

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YIELD AND VARIABILITY OF CHLOROPHYLL-MUTANT HETEROZYGOTES IN BARLEY

By *HANS DOLL*

AGRICULTURAL RESEARCH DEPARTMENT, DANISH ATOMIC ENERGY COMMISSION, RESEARCH ESTABLISHMENT RISØ, ROSKILDE, DENMARK AND DEPARTMENT OF GENETICS, ROYAL VETERINARY AND AGRICULTURAL COLLEGE, COPENHAGEN, DENMARK

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I. INTRODUCTION

AS yet no general agreement has been attained as to the extent to which recessive lethal genes have quantitative effects in the heterozygous condition. It is of special interest to know how often the heterozygote for a recessive lethal is superior to the wild-type homozygote, a condition which will here be termed *overdominance*. If overdominance occurs with a considerable frequency, this may for a great part account for the heterosis detected in many organisms, and also to a considerable extent for the genetic variability maintained by many natural populations.

A number of investigations concerning the viability, yield, variability, or other quantitative traits of heterozygotes for recessive lethal genes have been carried out; for a survey see FALK, RAHAT and BEN-ZEEV (1965) and HAGBERG (1953). Some of these investigations have dealt with lethal genes in plants, *e.g.* barley (GUSTAFSSON, 1946, 1947 and 1953; GUSTAFSSON *et al.*, 1950; ROBERTSON and AUSTIN, 1935), maize (MANGELSDORF, 1928), tomato (NILSSON, 1963), and a few other species. An outstanding feature of the plant studies is that overdominance has been found relatively often. This was for instance the case in most of the investigations carried out by GUSTAFSSON, and in similar studies of recessive genes in *Antirrhinum majus* (STUBBE and PIRSCHLE, 1940; STUBBE, 1953). However, overdominance has not invariably been found for all lethal genes studied in plants, and the total number of genes examined is modest. It is therefore difficult to judge how often and to what extent lethal genes in plants are beneficial in the heterozygotes.

In the investigation reported on here, the heterozygous effect of a random sample of recessive chlorophyll-mutant genes in barley was studied. The character *number of kernels per plant* was used in the comparison of the individual heterozygotes with the corresponding wild-type homozygotes. This trait constitutes an important component of yield and is closely related to the fitness or selective value of the plants.

Material and methods

49 chlorophyll-mutant genes from the two-rowed spring-barley variety *Carlsberg II* were initially available in the segregating offspring of 49 heterozygous plants. The individual offspring lines are denoted *mutant lines*. Each mutant line, which represents a particular chlorophyll-mutant gene, consists of mutants, heterozygotes and normal homozygotes in the ratio 1:2:1, provided the mutants segregate normally. The mutants and the lines are designated by the numbers 1 to 10 and 13 to 51.

A. *The mutants and their origin*

With the naked eye all mutant heterozygotes are phenotypically indistinguishable from the corresponding homozygous wild-type plants. Qualitatively, the mutant genes are thus completely recessive. With one exception, all the genes are homozygously lethal at the seedling stage. Homozygous plants of one mutant, no. 34, usually survive to ripening, but show reduced vigour.

On the average for all 49 mutants, heterozygous plants segregated approximately 22 per cent mutant homozygotes. Two of the mutants, nos. 24 and 33, segregated with a frequency of only approximately 10 per cent, nine mutants, nos. 5, 9, 14, 18, 21, 35, 36, 39, and 40, had segregation frequencies between 15 and 20 per cent, while the remaining 38 mutants did not differ substantially from the Mendelian expectation of 25 per cent.

When phenotypically classified according to GUSTAFSSON's system (GUSTAFSSON, 1940), the mutants comprise 34 *albinae*, 4 *virides*, 5 *xanthae*, and 6 of more infrequent types (see Table 2).

The heterozygous plants from which the mutant lines originated were found in an investigation of radiosensitivity in barley (FRYDENBERG and SANDFÆR, 1964). From each of three recurrently irradiated barley populations having received, during the years 1958 through 1961, total doses of 0. 25 and 50 krad respectively, four lots of kernels were selected

TABLE 1. *Distribution of the 49 heterozygous plants from which the mutant lines were derived, according to population of origin and according to the dose of irradiation they received in 1962.*

Original populations		Number of plants		Total number of plants
No.	Dose of irradiation 1958–61 krad	Irradiated with 0 krad in 1962	Irradiated with 10 krad in 1962	
1	0	1	1	2
2	25	10	12	22
3	50	9	16	25
Total number		20	29	49

in 1962 and irradiated with 0, 10, 17, and 24 krad γ-rays respectively. Afterwards the material was grown in the field as single plants spaced 25×40 centimetres apart.

Among the plants treated with 0 and 10 krad, 68 were found to be heterozygous for a chlorophyll mutant. The offspring kernels of 49 of these heterozygotes, *viz.* all those which had more than 200 kernels left after the offspring test, were used in the present investigation. Table 1 shows the distribution of the 49 heterozygous plants according to the dose of irradiation received in 1962 and according to their populational origin. It is seen from Table 1 that two of the mutants were spontaneous since they originated from the non-irradiated population. The other 47 mutants, which came from the irradiated populations, were presumably induced by the γ-irradiation.

B. *Experimental methods*

To avoid spacing heterogeneity due to non-viable mutant plants, the mutant-line kernels were sown individually, surrounded by common plants of *Carlsberg II* (see Fig. 1). As a general rule, 405 kernels were sown from each mutant line. Owing to lack of material, only 324 kernels were sown of lines nos. 48 and 49, 270 kernels of no. 50, and 216 of no. 51.

After harvest, the numbers of kernels and of sterile flowers were registered for the individual mutant-line plants. During the following winter, the genotypes of the plants were determined by germinating, if available, 20 kernels from each plant in a greenhouse. Segregating plants were considered heterozygous. Non-segregating plants were classed as

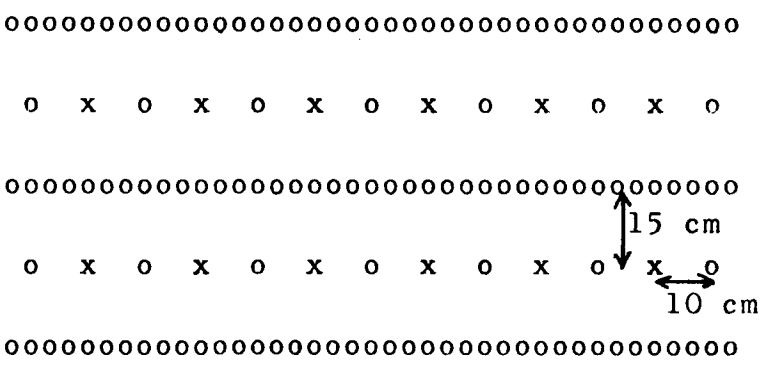

Fig. 1. The layout of the material in the experimental field. x: plant of mutant line sown by hand. o: two common barley plants sown by hand in one place. ooo: common barley sown by machine in rows with about 35 plants per metre.

homozygous wild-types unless they belonged to a mutant line in which the heterozygotes segregated less than 15 per cent mutants. If so, another 20 kernels were tested, if available, in order to avoid misclassification.

C. *Statistical methods*

Within each mutant line the heterozygous and the wild-type homozygous plants were compared with respect to *mean* and *variability* of the number of kernels per plant. As a measure of the difference between the means of the two genotypes in a mutant line, a *dominance index*, D, was defined by the expression

$$D = (\bar{x}_{Aa} - \bar{x}_{AA}) / (s^2_{Aa}/n_{Aa} + s^2_{AA}/n_{AA})^{\frac{1}{2}},$$

where $\bar{x}$, s^2 and n signify mean, variance and number of plants, respectively, and Aa symbolizes the heterozygous and AA the homozygous plants. It is seen that the dominance index is the standardized difference between the means of the two genotypes, *i.e.* the difference measured in units of standard errors. A positive dominance index reveals that the heterozygote was superior to the homozygote, while a negative index indicates that the heterozygote was the inferior one. Provided the true mean of the heterozygous plants was not different from that of the homozygous plants, this index is approximately normally distributed, with a mean of zero and unit variance (HALD, 1952 a).

To judge whether the aggregate of the observed dominance indices showed that the two compared genotypes differed in at least some of the mutant lines, the following statistics were evaluated (HALD, 1952 a):

$$u=\sum_{i=1}^{N} D_i/\sqrt{N} \quad \text{and} \quad \chi^2=\sum_{i=1}^{N} (D_i-\overline{D})^2.$$

N is the number of indices and $\overline{D}$ the mean of these. If all true indices are zero, u is a standardized normal variable, and a significant u-value indicates that, on the average, the mutant genes have affected the mean of the heterozygous plants. Provided all true indices are the same, though not necessarily zero, χ^2 has a χ^2-distribution with N-1 degrees of freedom. Hence a significant χ^2-value indicates a different heterozygous effect of the genes.

To evaluate whether any of the individual indices could be declared significantly different from zero, bilateral 5 per cent limits of significance were chosen as follows. Let p be the probability that a single standardized, normal variable falls within a given central interval of the distribution. The probability that none of N such variables fall outside this interval is then p^N. This probability is wanted to be equal to 95 per cent, and we have $p^N=0.95$. For $N=49$, p becomes 0.99895, which corresponds to the interval ± 3.28 in a standardized normal distribution (HALD, 1952 b). The limits of this interval constitute the appropriate 5 per cent significance limits when an aggregate of 49 indices are tested simultaneously.

The variability of a genotype was expressed by the coefficient of variation, $c=s/\bar{x}$, which is approximately normally distributed, with the variance (HALD, 1952 a)

$$V\{c\}=(c^2+2c^4)/2f,$$

where f denotes the degrees of freedom of s.

For each mutant, the *dominance index* with respect to *variability* was defined by

$$D=(c_{Aa}-c_{AA})/(V\{c_{Aa}\}+V\{c_{AA}\})^{\frac{1}{2}},$$

i.e. the standardized difference between the coefficients of variation of the two genotypes. If the two coefficients compared are truly identical, this index is approximately normally distributed, with a mean of zero and unit variance. The tests of significance already described have therefore also been applied to the variability indices.

TABLE 2. *Mean and variability of the number of kernels for the two normally green genotypes in each mutant line.*

The symbols *AA* and *Aa* denote the normal wild-type and the mutant heterozygote respectively.

Mutant no. and type[1]	Number of plants		Mean			Variability		
			Average number of kernels		Dominance index	Coefficient of variation		Dominance index
	Aa	AA	Aa	AA		Aa	AA	
1-A	187	100	80.1	76.2	+ 1.05	0.413	0.362	+ 1.32
2-A	188	92	78.8	82.7	− 0.94	0.387	0.415	− 0.65
3-A	164	85	71.6	63.6	+ 2.24	0.405	0.397	+ 0.18
4-A	183	96	70.9	81.7	− 2.79	0.411	0.389	+ 0.54
5-A	198	123	67.0	64.8	+ 0.78	0.390	0.371	+ 0.56
6-A	198	74	68.8	73.5	− 1.23	0.395	0.390	+ 0.11
7-A	196	90	73.5	67.7	+ 1.54	0.351	0.456	− 2.31
8-A	175	82	62.1	64.3	− 0.56	0.409	0.477	− 1.31
9-X	192	104	57.1	56.1	+ 0.28	0.480	0.484	− 0.07
10-A	191	73	73.6	77.0	− 0.76	0.429	0.424	+ 0.11
13-V	194	97	53.8	55.0	− 0.45	0.395	0.389	+ 0.15
14-A	170	135	57.2	61.4	− 1.55	0.370	0.413	− 1.18
15-A	189	96	60.8	68.6	− 2.68	0.330	0.355	− 0.74
16-A	180	118	68.0	73.3	− 1.66	0.387	0.370	+ 0.47
17-A	166	127	62.3	77.2	− 4.61	0.371	0.394	− 0.63
18-A	170	117	54.2	63.0	− 2.82	0.449	0.427	+ 0.50
19-A	190	82	67.0	75.2	− 1.95	0.404	0.450	− 0.96
20-A	161	102	73.0	72.0	+ 0.28	0.413	0.422	− 0.21
21-A	162	83	58.8	57.8	+ 0.28	0.424	0.458	− 0.67
22-M	166	85	63.6	61.2	+ 0.73	0.427	0.382	+ 1.04
23-A	195	82	62.4	60.9	+ 0.47	0.407	0.397	+ 0.23
24-V	156	136	63.6	69.1	− 1.72	0.416	0.404	+ 0.30
25-MX	174	76	77.7	76.0	+ 0.37	0.399	0.454	− 1.09
26-X	153	69	72.5	70.4	+ 0.52	0.360	0.409	− 1.05
27-A	143	96	72.3	77.0	− 1.16	0.428	0.406	+ 0.50
28-A	153	100	71.1	66.2	+ 1.20	0.443	0.487	− 0.84
29-AV	159	89	60.3	67.4	− 1.69	0.469	0.496	− 0.50
30-A	146	78	61.6	68.7	− 1.73	0.423	0.446	− 0.44
31-A	170	84	80.6	76.4	+ 0.89	0.403	0.471	− 1.35
32-A	161	91	64.3	68.0	− 0.91	0.507	0.444	+ 1.21
33-A	138	137	58.0	62.2	− 1.36	0.417	0.428	− 0.26
34-V	153	90	59.1	61.8	− 0.75	0.499	0.433	+ 1.29
35-A	148	102	58.8	56.9	+ 0.58	0.422	0.428	− 0.13
36-AV	157	76	65.7	68.0	− 0.54	0.492	0.413	+ 1.53
37-A	139	91	60.4	62.2	− 0.46	0.496	0.459	+ 0.67
38-X	158	79	49.9	51.7	− 0.58	0.481	0.411	+ 1.40
39-A	139	103	62.4	65.7	− 0.89	0.415	0.466	− 1.05
40-A	134	128	43.2	42.4	+ 0.31	0.494	0.498	− 0.07

TABLE 2. *(Cont.)*

Mutant no. and type[1]	Number of plants		Mean			Variability		
			Average number of kernels		Dominance index	Coefficient of variation		Dominance index
	Aa	AA	Aa	AA		Aa	AA	
41-A	149	108	69.2	63.2	+ 1.43	0.440	0.565	− 2.15
42-A	167	84	63.6	62.9	+ 0.20	0.447	0.417	+ 0.64
43-AV	157	103	53.5	51.6	+ 0.66	0.446	0.428	+ 0.39
44-V	181	69	46.6	46.6	− 0.01	0.411	0.414	− 0.06
45-X	153	55	56.9	62.0	− 1.26	0.453	0.406	+ 0.88
46-A	171	70	48.6	46.6	+ 0.64	0.467	0.457	+ 0.18
47-AV	155	60	61.3	60.7	+ 0.13	0.456	0.543	− 1.24
48-A	118	66	54.3	51.7	+ 0.66	0.430	0.496	− 1.06
49-A	136	80	53.2	56.3	− 0.82	0.460	0.511	− 0.85
50-A	89	52	29.5	28.2	+ 0.48	0.602	0.479	+ 1.49
51-X	83	29	51.5	35.5	+ 3.41	0.459	0.599	− 1.24
Average	162	91	62.3	63.4	− 0.34	0.430	0.439	− 0.13

[1] Mutant types:
A: *albina*; X: *xantha*; AV: *alboviridis*;
V: *viridis*; M: *maculata*; MX: *maculata—xantha.*

II. RESULTS

The results obtained for the 49 mutants in 1963 are presented in Table 2. In 1964, 22 of the genes were tested again, and these results appear in Table 3. In the tables the mutants are designated by their number followed by a letter indicating the mutant type.

Being the offspring of a heterozygous plant, twice as many heterozygotes as dominant homozygotes are expected in the individual mutant lines. The actual number of plants (see Table 2) deviated considerably from this expectation in some of the lines, *e.g.* in nos. 24, 33 and 40, where the wild-types predominated. A study of the segregation of the mutants has shown that these deviations are most often due to reduced transmission of the mutant gene through the male gametophyte.

The fertility of the plants, as expressed by the frequency of seed setting, was also estimated, and some variation among the mutant lines was found. There were, however, only minor and insignificant differences in fertility between the two genotypes within mutant lines. The observations on fertility are not presented in detail as they seem to be of no significance.

TABLE 3. *Results of repeated testing in 1964 of the 22 mutant genes for which the heterozygote was superior to the wild-type homozygote in the main experiment in 1963.*

Mutant no. and type[1]	Number of plants		Mean			Variability		
			Average number of kernels		Dominance index	Coefficient of variation		Dominance index
	Aa	AA	Aa	AA		Aa	AA	
1-A	192	104	112.7	111.3	+ 0.35	0.288	0.302	− 0.51
3-A	187	111	100.2	97.8	+ 0.68	0.298	0.288	+ 0.38
5-A	196	131	98.8	98.8	0.00	0.276	0.263	+ 0.57
7-A	185	102	104.2	109.8	− 1.44	0.306	0.283	+ 0.85
9-X	200	109	89.3	91.0	− 0.52	0.313	0.301	+ 0.43
20-A	202	101	88.8	86.7	+ 0.82	0.251	0.245	+ 0.26
21-A	185	128	97.6	102.3	− 1.45	0.278	0.288	− 0.39
22-M	191	108	93.8	95.4	− 0.51	0.289	0.275	+ 0.54
23-A	168	89	101.3	100.2	+ 0.28	0.321	0.274	+ 1.60
25-MX	181	118	114.1	117.9	− 0.97	0.275	0.285	− 0.38
26-X	177	106	98.0	96.2	+ 0.45	0.322	0.341	− 0.59
28-A	196	105	92.7	98.1	− 1.58	0.335	0.271	+ 2.32
31-A	159	77	105.8	103.0	+ 0.70	0.250	0.293	− 1.45
35-A	195	119	89.0	85.5	+ 1.20	0.275	0.301	− 1.02
40-A	161	142	59.2	62.8	− 1.44	0.344	0.366	− 0.71
41-A	198	98	82.4	89.6	− 2.10	0.324	0.321	+ 0.11
42-A	202	97	95.6	94.3	+ 0.39	0.276	0.279	− 0.11
43-AV	194	107	98.0	96.9	+ 0.35	0.259	0.251	+ 0.33
46-A	183	82	88.4	97.3	− 2.61	0.293	0.265	+ 1.01
47-AV	184	100	86.3	81.5	+ 1.66	0.253	0.294	− 1.55
48-A	201	99	86.1	91.5	− 1.61	0.305	0.307	− 0.07
50-A	173	100	65.7	73.7	− 1.94	0.473	0.467	+ 0.11
Average	187	106	93.1	94.6	− 0.42	0.300	0.298	+ 0.08

[1] See note to Table 2.

1. The mean number of kernels

A. *The main experiment in 1963*

A grouped distribution of the mutants according to their dominance index (see methods) is shown in Fig. 2, in which one half standard error is used as class interval. A normal distribution curve with a mean of zero and unit variance is superimposed on the diagram, indicating the expected distribution of the dominance indices provided these deviate only at random from zero, *i.e.* provided the observed differences be-

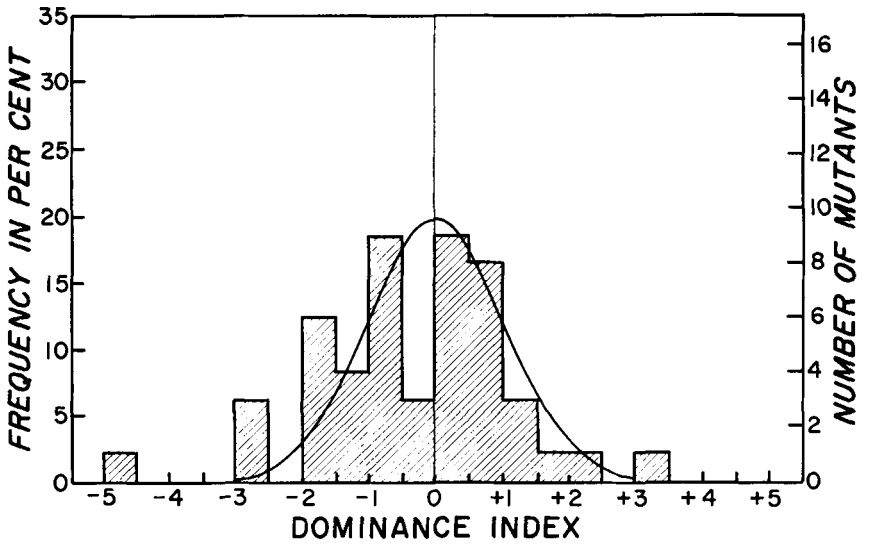

Fig. 2. Grouped distribution of the mutants according to dominance index with respect to mean yield in the main experiment in 1963. The smooth curve is the expected distribution of a normal variable with a mean of zero and unit variance.

tween the two genotypes are due exclusively to sampling in all the mutant lines.

To examine whether the material as a whole shows any significant deviations from the zero hypothesis stated, the two tests of significance described under Statistical methods are applied. The first of these serves to show whether the mutant genes have on the average affected the yield of the heterozygotes significantly. This test gives a standardized normal variable of 2.39, which, in a two-sided test, corresponds to a significance probability of 0.017. The second test, which judges the significance of the difference between the effects of the genes, gives $\chi^2=96.58$, $d.f.=48$, $P < 0.0005$. From these results it is concluded that at least some of the mutant genes have affected the average number of kernels of the heterozygous plants.

According to their heterozygous effect on the yield, the mutant genes may be of three types, which are detrimental, neutral and overdominant respectively. The two tests applied above show that not all the genes studied are of the neutral type. As the mean of the dominance indices is negative, $\overline{D}=-0.34$ (Table 2, bottom), some of the genes must be of the detrimental type. However, the tests do not give very much informa-

TABLE 4. *Classification of the 49 dominance indices from the main experiment into three groups with the same number of indices expected in each.*

	Limits[1] of the groups		
	$-\infty$ to -0.43	-0.43 to $+0.43$	$+0.43$ to $+\infty$
Number of indices expected	16.33	16.33	16.33
Number of indices observed	25	8	16
Chi-square deviation	4.603	4.249	0.007
$\chi^2 = 8.86$; 2 degrees of freedom; $P < 0.025$			

[1] Calculated by means of a table of the cumulative normal distribution function (HALD, 1952b).

tion about the true distribution of the genes on the three types mentioned above.

To investigate this point further, the dominance indices have been classified into three groups chosen so that they have the same expected number of indices if all the genes are neutral. The grouping is given in Table 4, in which the distribution observed is compared with that expected by a chi-square goodness-of-fit test. It appears that the observed number of dominance indices in the negative group is much higher than expected, while there is a marked deficit in the central group. These deviations are significant at the 2.5 per cent level (Table 4), and it is concluded that a considerable number of the genes have a true detrimental effect. When this is the case, the expected number of dominance indices in the positive group is less than or equal to that in the central group provided no genes are of the overdominant type. The fact that twice as many dominance indices fall in the positive as in the central group suggests that some of the genes have a true positive heterozygous effect on the mean yield. However, this suggestion is difficult to test since the real number of detrimental mutant genes is unknown.

To settle whether the effect of any of the individual mutant genes was significant, the dominance indices have been compared with the limits -3.28 and $+3.28$, which correspond to the 5 per cent level of significance when 49 indices are tested simultaneously (see methods). It appears from Table 2 and Fig. 2 that the indices -4.61 and $+3.41$, belonging to mutants nos. 17 and 51 respectively, fall outside the critical

limits. These two genes are the only ones which, when evaluated individually, have a demonstrable significant effect on the average number of kernels.

Little interest attaches to the mutant gene no. 17, which is but the most deviating member of the group of genes with detrimental effect. The significance found in line 51 is more interesting since it indicates the occurrence of overdominance. However, this line was also segregating for another recessive gene which is linked in repulsion to the mutant gene and which causes necrotic spots on the plants and reduces their yield considerably. This linkage may be responsible for the overdominance observed. A more detailed investigation of this mutant line is in progress, but has not yet clarified the nature of the overdominance.

The experiment in 1963 established the facts that the heterozygous effect on yield of the 49 chlorophyll-mutant genes varied, and that a considerable number of the genes had a more or less pronounced negative effect, which led to a negative average effect. It appears from the bottom line in Table 2 that this negative average effect is of the order of 2 per cent. Apart from the special case of mutant gene no. 51, no significant indication could be found of some of the genes having increased the kernel yield in the heterozygote.

B. *The retests in 1964*

In an attempt to isolate possible overdominant mutant genes, the 23 mutants that had had a positive dominance index in the main experiment were retested in an experiment in the following year. The 405 kernels sown of each mutant line in 1964 were the offspring of from 5 to 15 heterozygous plants from 1963.

The results of the retests are presented in Table 3, and a grouped distribution of the dominance indices is shown in Fig. 3. One of the 23 retested genes was no. 51, which for special reasons showed overdominance both in the main experiment and in the retest. In the following we are only concerned with the 22 other mutants, and the results of the retest of no. 51 are not given in Table 3.

If, for a considerable number of the lines, the positive dominance index found in 1963 was the manifestation of a true positive index, one would expect that the estimates obtained in 1964 would also tend to be positive. But a comparison with the expected random distribution in Fig. 3 does not reveal that the observed distribution is displaced towards the positive side. Of the 22 indices just 11 fall above and 11 below zero. Further there is a tendency to an excess in the negative intervals from

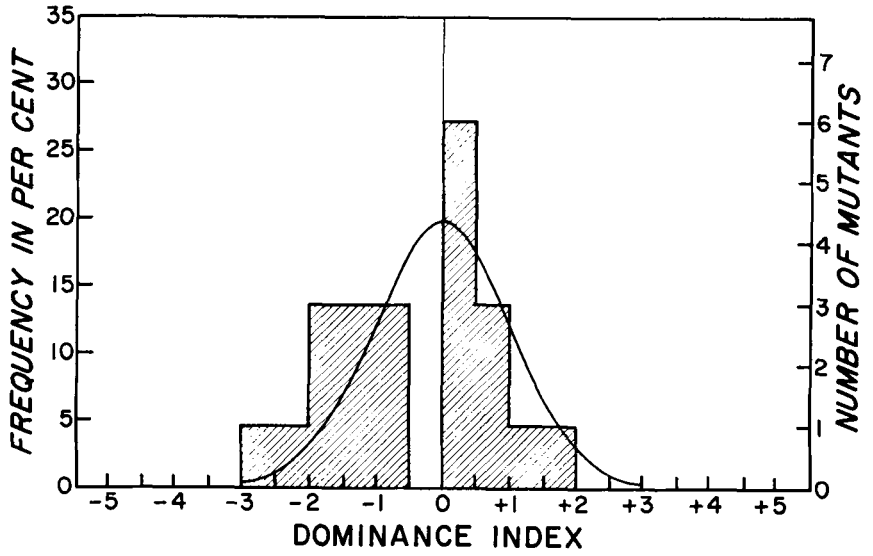

Fig. 3. Grouped distribution of the 22 retested mutants according to dominance index with respect to mean yield. The smooth curve is the expected distribution of a normal variable with a mean of zero and unit variance.

-3.00 to -1.00, a fact that also contributes to the negative mean of the dominance indices, $\overline{D}=-0.42$ (Table 3, bottom).

The deviation of $\overline{D}$ from zero is just significant at the 5 per cent level ($u=-1.98$, $P=0.048$, two-sided test), so on the average the 22 mutant genes have affected the yield significantly in a negative direction. The chi-square test of the variation between the effects of the individual genes yielded $\chi^2=30.37$, $d.f.=21$, $0.05<P<0.10$. Though insignificant, some variation in the effects of the different genes is indicated.

When the dominance indices are classified into three groups with equal numbers expected in them (Table 5), a surplus of mutants with a high negative dominance index becomes evident once more; the largest observed number is again found in the group from $-\infty$ to -0.43. However, the goodness-of-fit χ^2-value is insignificant, so the deviations might be due to sampling alone. The limits of significance corresponding to the 5 per cent level for the individual dominance indices (see methods) are calculated to be ± 3.04. None of the mutants in Table 3 has an index outside these limits.

The failure of the tests to demonstrate any overdominant mutant gene among the 22 retested ones may be due to the limited power of

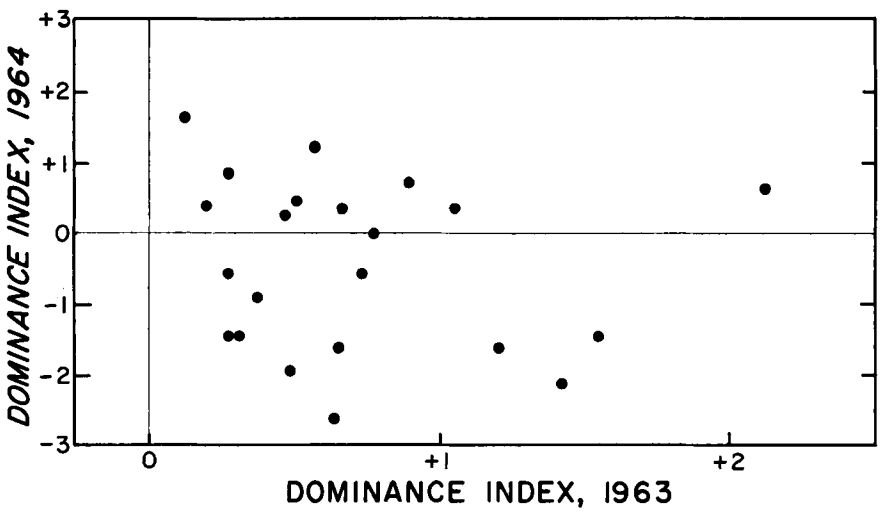

Fig. 4. Relationship between the 22 dominance indices which were estimated both in 1963 and in 1964.

the tests, and it is therefore important to investigate, pair by pair, the association between the 22 dominance indices estimated in both 1963 and 1964. On the assumption that the effect of a gene does not depend entirely on the year in which it is tested, a beneficial effect should result in a positive association. The relationship between the results of the two years is shown in Fig. 4. It is obvious from this figure that no positive association exists between the two sets of dominance indices, and this is confirmed by a regression coefficient that is negative, $b = -0.28$,

TABLE 5. *Classification of the 22 dominance indices from the retests into three groups with the same number of indices expected in each.*

	Limits[1] of the groups		
	$-\infty$ to -0.43	-0.43 to $+0.43$	$+0.43$ to $+\infty$
Number of indices expected	7.33	7.33	7.33
Number of indices observed	11	5	6
Chi-square deviation	1.837	0.741	0.241
$\chi^2 = 2.82$; 2 degrees of freedom; $0.20 < P < 0.30$			

[1] See the note to Table 4.

though not significantly different from zero. Everything considered, there is thus nothing in the data to indicate that any of the 22 retested chlorophyll-mutant genes were overdominant.

2. The variability of the number of kernels

Besides their heterozygous effect on the mean yield, the 49 mutant genes may also have had an influence on the yield variability of the heterozygotes. In the present case, the standard deviation is an unsuitable measure of the variability in number of kernels since it is positively related to the mean of that number. The standard deviation would thus give an average-dependent measure of the variability. However, the standard deviation and the mean are approximately proportional to each other so that the coefficient of variation will be independent of the average. This quantity is therefore chosen to express the variability of the plants.

The coefficients of variation of the two genotypes within each mutant line appear in Tables 2 and 3 for the main experiment and the retests respectively. Further the tables give the dominance indices of the mutants with respect to variability, *i.e.* the standardized difference between the coefficients of variation of the genotypes (see methods). The average coefficient of variation was 0.435 in 1963 and 0.299 in 1964. The difference was probably a pure year effect since the experiments were carried out identically.

The distributions of the dominance indices with respect to variability in 1963 and 1964 are shown in Figs. 5 and 6, which are similar to Figs. 2 and 3. Apart from a weak tendency towards a negative shift in 1963, the two observed distributions of the indices agree well with those expected if there is no real difference in variability between heterozygotes and homozygotes.

The hypothesis that the two sets of dominance indices only deviate randomly from zero may be tested by means of the two tests of significance employed earlier. The test concerning the average of the 49 dominance indices found in 1963 gives $u=-0.92$, $P=0.36$, while the test evaluating the homogeneity of these indices yields $\chi^2=42.23$, $d.f.=48$, $0.70<P<0.80$. The corresponding results for the 22 dominance indices from the retests are: $u=+0.37$, $P=0.71$, and $\chi^2=17.67$, $d.f.=21$, $0.60<P<0.70$. Since both the u- and the χ^2-values are insignificant in both experiments, the differences between the coefficients of variation of the heterozygous and the homozygous genotypes are no greater

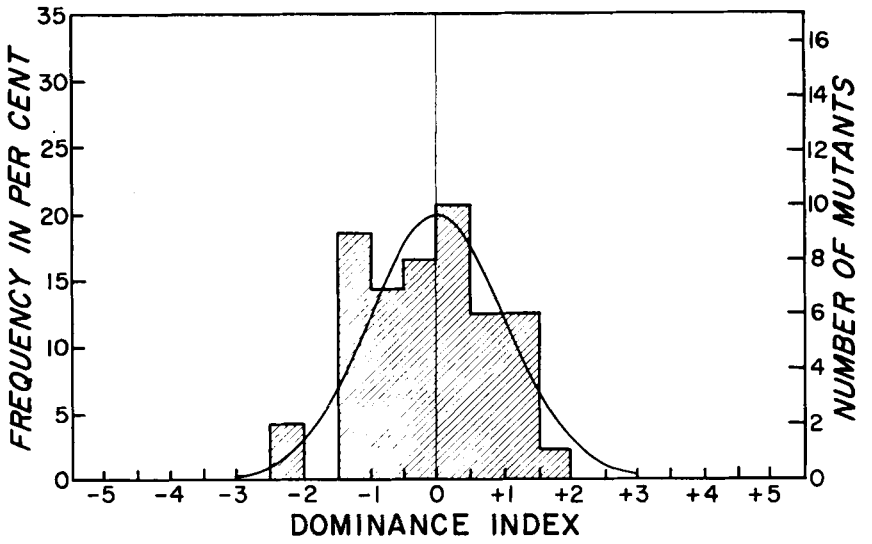

Fig. 5. Grouped distribution of the mutants according to dominance index with respect to variability in the main experiment. The smooth curve is the expected distribution of a normal variable with a mean of zero and unit variance.

than expected from sampling. Since, furthermore, the mean dominance indices from the two years have different signs, there is nothing at all to indicate that the chlorophyll-mutant genes have affected the variability of the number of kernels in the heterozygous plants.

III. DISCUSSION

1. The mean yield

Generalizing the results presented, one seems justified in drawing the following three conclusions concerning the heterozygous effect of chlorophyll-mutant genes on the mean kernel yield. First, a majority of the genes have no or very little effect; secondly, a not inconsiderable fraction have a distinct detrimental effect. Thirdly, overdominant genes occur very rarely. Averaged over all mutants, the kernel yield of the heterozygotes is approximately two per cent less than that of the corresponding wild-type homozygotes.

These conclusions are almost similar to those drawn in most investigations of recessive lethals in *Drosophila melanogaster*. STERN, CARSON, KINST, NOVETSKI, and UPHOFF (1952) examined 75 spontaneous

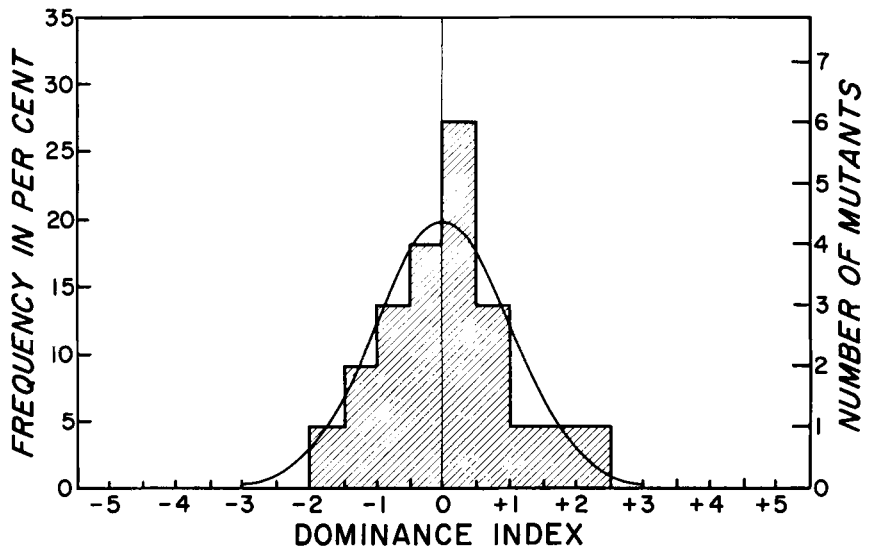

Fig. 6. Grouped distribution of the 22 retested mutants according to dominance index with respect to variability. The smooth curve is the expected distribution of a normal variable with a mean of zero and unit variance.

and induced sex-linked lethals, which, on the average, were found to reduce the viability of their heterozygous carriers by 4 per cent. A similar average detrimental effect has been demonstrated repeatedly, *e.g.* by HIRAIZUMI and CROW (1960) and by FALK, RAHAT and BEN-ZEEV (1965), who studied respectively autosomal recessive lethals isolated from nature and induced, sex-linked recessive lethals. Though a few cases of overdominance were found by STERN *et al.* (1952), these investigations showed quite clearly that recessive lethals generally have a deleterious effect in the heterozygous condition. However, it should be mentioned that for instance WALLACE (1958, 1965) has claimed that lethals in *Drosophila* are neutral or even advantageous when heterozygous.

In a study of heterozygous effect in yeast, JAMES (1960) also found little evidence of overdominance. The mean effect of 35 recessive lethals and near lethals on the growth rate of heterozygotes was a reduction of eight per cent. However, the majority of the mutant genes had no or little effect since the modal value of the heterozygotes was within two per cent of normal.

Some of the earlier investigations on chlorophyll mutants also show

results compatible with the present ones. ROBERTSON and AUSTIN (1935) examined three spontaneous barley mutants and found that they had no detrimental heterozygous effect. Similar conclusions have been drawn in three other investigations (KARPER, 1930; ROBERTSON, 1932; NILSSON, 1963). Since all these authors worked with a small sample of different mutants, it is quite reasonable that they only recognized the majority group of genes with little or no heterozygous effect.

The results of other investigations of chlorophyll mutants are, however, more at variance with the present ones. Thus, in *Anthirrhinum majus*, STUBBE and PIRSCHLE (1940) and STUBBE (1953) observed that plants which were heterozygous for one or two chlorophyll-mutant genes were, in general, clearly superior in vigour to normal homozygous plants, and no indications were found that some genes had harmful effects on the heterozygote. Similarly, GUSTAFSSON (1946, 1947, 1952, 1953; GUSTAFSSON *et al.*, 1950) has demonstrated convincingly that heterozygotes for one or two of the mutant genes studied had regularly a considerably higher yield than normal homozygotes.

Several reasons may be suggested for the apparent discrepancy between the above-mentioned results and those put forward in this paper. While our mutants come close to being a random sample of induced chlorophyll mutants, those of GUSTAFSSON and co-workers and of STUBBE and PIRSCHLE may have been less representative. Actually it seems doubtful whether these authors aimed at examining random samples of mutants.

Another possibility to be considered is that the heterozygous effect of a given mutant gene may vary with the environmental test conditions. This was demonstrated by GUSTAFSSON *et al.* (1950), who compared the genotypes at varying amounts of nitrogen fertilizer and at different plant densities. The beneficial effect of the genes changed considerably with growing conditions and was at some combinations of the two factors converted into a detrimental effect. However, different genes attained their optimum effect under different conditions, and it seems improbable that the experimental conditions in the present investigation have been so unique as to be the reason why no cases of true overdominance were found.

Finally, the possibility cannot be neglected that the discrepancy between GUSTAFSSON's and STUBBE's results and the present ones is due to the different ways in which the experiments were designed. The combined effect of two or more pairs of genes may imitate overdominance even though none of the pairs has this property *per se*. When it is in-

tended to examine a single locus, it is therefore of the utmost importance to ensure that the genotypes compared differ only with respect to the two alleles in question. GUSTAFSSON (1946) has pointed out that this requirement is most easily met when one of the alleles tested has arisen by mutation in a homozygous material, *e.g.* lines of barley and other self-fertilizing plants. However, even though self-fertilizing organisms are favourable, they may contain disturbing heterozygosity in unknown loci due to crossing or mutation.

The mutant lines comprised by the present investigation were derived directly from a heterozygote removed one or a few generations of self-fertilization from the mutational event. No artificial crossing intervened between the latter and the testing of the mutant. In most of the cited investigations of chlorophyll mutants the test material was the segregating offspring of dihybrid heterozygotes produced by crossing two different mutant lines. Though these lines originally came from the same variety, the isolation and the subsequent crossing may have led to heterozygosity in loci affecting the characters studied. If such loci were linked with the one tested, they may have caused a multi-genic heterosis undistinguishable from the effect of the individual chlorophyll-mutant genes. As pointed out by MÜNTZING (1945), objections of this kind may be made especially against the method used in the work of STUBBE and PIRSCHLE (1940) and STUBBE (1953). These authors examined non-lethal mutant genes by crossing a homozygous mutant line with the normal line in which the mutant had originated, and then compared the F_1 with the normal line. Obviously the F_1 plants were heterozygous in all loci in which the mutant line had diverged from its parental line. This procedure allows the disturbing influence not only of possible heterozygous loci linked to the chlorophyll-mutant gene examined, but also of heterozygous loci in other chromosomes.

2. The variability

This investigation did not indicate any difference in variability between chlorophyll-mutant heterozygotes and wild-type homozygotes. It has often been claimed that heterozygosity is accompanied by greater phenotypic stability than found in comparable homozygotes. This has for instance been demonstrated in *Drosophila pseudoobscura* by DOBZHANSKY and LEVENE (1955) and in maize by SHANK and ADAMS (1960). These organisms are, however, cross-fertilizers, and LERNER (1954) has pointed out that self-fertilizers may be different from cross-fertilizers

since natural selection acts preferably on respectively homozygous and heterozygous individuals in the two forms. But ALLARD and WORKMAN (1963), working with lima beans, and GRIFFING and LANGRIDGE (1963), working with *Arabidopsis thaliana*, reached the conclusion that also these self-fertilizers formed heterozygotes which, mainly because of superiority in extreme environments, had a greater all-over phenotypic stability than homozygotes.

However, in these cases it may be assumed that a number of loci were heterozygotic simultaneously, and the observed stability may be a pseudo-stability brought about by the fact that the homozygotes involved were homozygous for different partly recessive genes. In the present experiment there is nothing to indicate that the wild-type homozygotes should have been homozygous more often in irrelevant loci than the chlorophyll-mutant heterozygotes. Presumably we have been close to comparing the mutant heterozygotes with wild-type homozygotes that were otherwise genetically identical with the heterozygotes.

In contrast to the above, GUSTAFSSON (1946, 1947) claimed that chlorophyll-mutant heterozygotes were less stable, *i.e.* varied more, than wild-type homozygotes. With the variability expressed as the coefficient of variation it was demonstrated that the two mutants *albina 7* and *xantha 3*, when heterozygous, increased the variability of the number of spikes, the number of kernels, and the weight of kernels per plant. GUSTAFSSON (1946) furthermore concluded from the data given by ROBERTSON (1932) and ROBERTSON and AUSTIN (1935) that the chlorophyll-mutant heterozygotes investigated by these authors generally also varied more than the corresponding dominant homozygotes.

In order to see whether ROBERTSON and AUSTIN's data carry significant evidence to the view that heterozygotes are more variable than homozygotes, the coefficients of variation and the dominance indices have been calculated from their published results and tested in the same way as in the present investigation. The study comprised five different monohybrid heterozygotes and two of the possible dihybrid heterozygotes between the five mutant genes. The variability dominance indices are shown in Table 6 for each of the five characters measured. Where several sets of data are available for a given mutant, Table 6 gives the combined dominance index, which has been calculated from the formula for u mentioned under methods. When a total of 35 indices are tested simultaneously, the proper 5 per cent limits of significance are ± 3.18. None of the dominance indices in Table 6 exceed these limits. The tests of the material as a whole give: $u = +1.79$, $P = 0.07$ and $\chi^2 =$

TABLE 6. *Dominance indices with respect to variability of mono- and dihybrid heterozygotes for chlorophyll-mutant genes in barley.*

The indices have been calculated from the data given by ROBERTSON (1932) and ROBERTSON and AUSTIN (1935).

Type of mutant indicated by origin variety	Characters				
	Weight of grains	Number of grains	Length of culm	Length of head	Culms per plant
Black Hulless II	+ 0.63	+ 0.96	− 0.16	+ 1.30	+ 0.52
Hanna	+ 0.96	+ 0.23	− 0.32	+ 0.05	+ 1.87
Colsess I	− 1.32	− 0.68	− 0.44	+ 0.40	− 0.69
Colsess II	− 0.02	+ 0.70	+ 0.35	+ 0.43	+ 0.34
Colsess IV	+ 0.83	− 0.16	− 1.56	+ 0.36	+ 1.03
Colsess I—Colsess IV	+ 0.05	+ 0.17	+ 0.31	+ 0.18	+ 0.26
Colsess II—Colsess IV	+ 0.34	+ 1.53	− 0.09	+ 2.06	+ 0.18

$=19.39$, $d.f.=34$, $0.950 < P < 0.975$. The positive deviation of the mean value from zero is nearly significant at the 5 per cent level. However, since the χ^2-value found is significantly too *low*, the variation between the 35 indices is smaller than expected from sampling, probably because the five properties measured are more or less positively correlated. If this is the case, the u-test of the mean value will tend to overestimate the significance. When this is taken into consideration, these data do not seem to indicate a significantly higher variability of the chlorophyll-mutant heterozygotes investigated by ROBERTSON and AUSTIN.

It is not possible to make similar calculations and tests on the results of GUSTAFSSON's own investigations. However, it must be admitted that the indications of a higher heterozygous variability in that material are rather strong. No reason for the discrepancy between GUSTAFSSON's and the present data will be suggested, but it may be of significance that the two groups of mutant genes differed in their effect on the mean yield, the present being, on the average, detrimental while those investigated by GUSTAFSSON and co-workers were generally associated with over-dominance.

SUMMARY

The heterozygous effect in barley of a random sample of recessive chlorophyll-mutant genes was studied. The investigation comprised 49 genes which came from γ-irradiated barley populations and, with one

exception, were lethal when homozygous. The mean and variability of the number of kernels per plant in wild-type homozygotes and in heterozygotes were examined.

On the average, the tested sample of mutant genes reduced the mean number of kernels of the heterozygous plants by approximately two per cent. However, the mutant sample was heterogeneous: some of the genes had apparently little or no effect in the heterozygous condition, while others had a more or less pronounced detrimental effect on their heterozygous carriers. One of the mutant heterozygotes had a significantly larger mean number of kernels than the corresponding homozygous wild-type. However, this particular mutant gene was found to have been linked in repulsion to another recessive mutant gene. Until further results are available, this linkage is assumed to be responsible for the overdominance.

By repeated testing of a selected group of the mutants, an attempt was made to isolate genes with a beneficial heterozygous effect, but no indication of true overdominance was found for any of the mutants tested.

The coefficient of variation was used to express the variability of the plants. On the average the tested sample of mutant genes had no significant effect on the variability of the heterozygous plants, and no variation between the effects of the individual genes on this trait could be demonstrated.

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